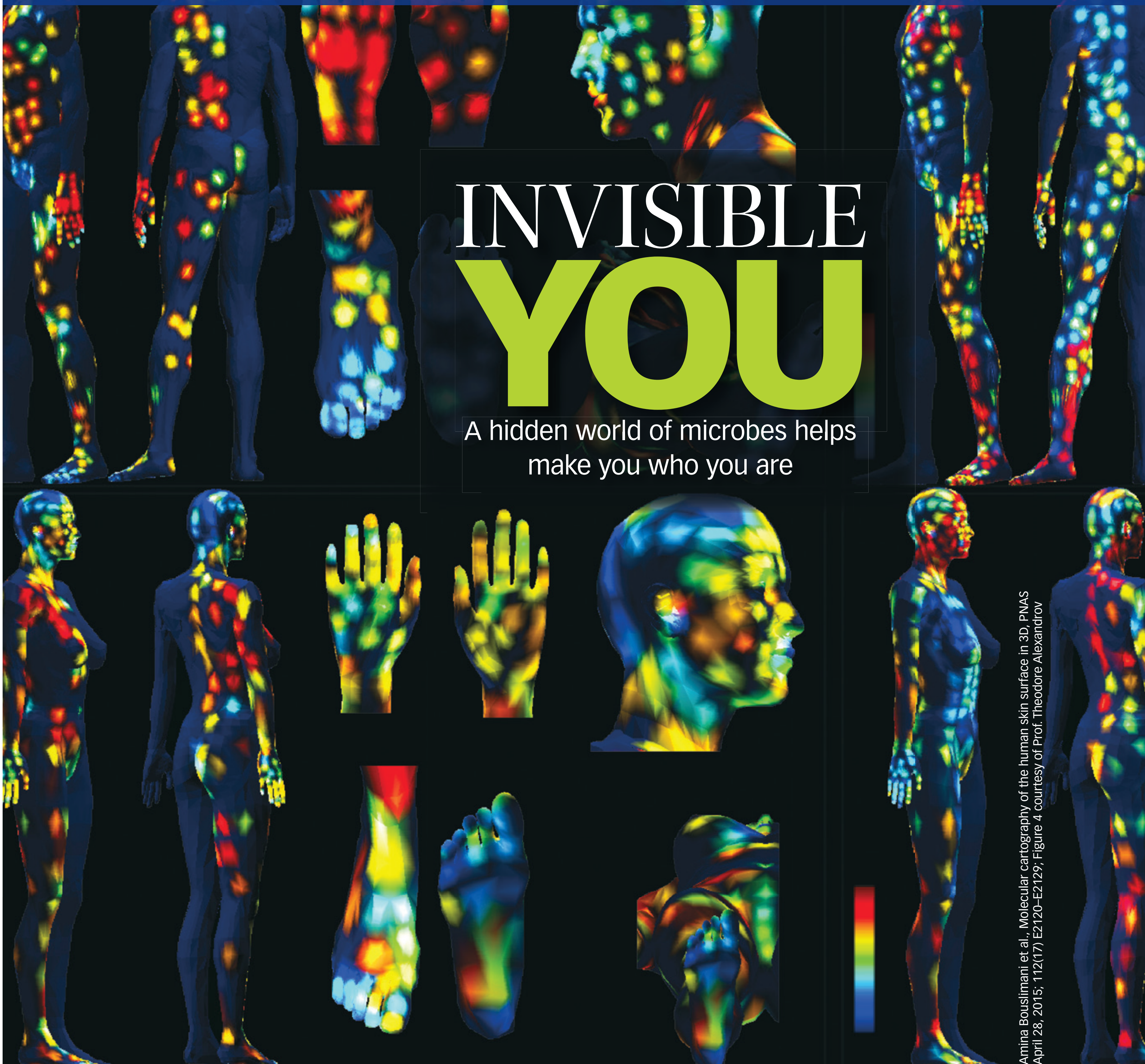
INVISIBLE YOU

A hidden world of microbes helps make you who you are



Amina Bouslimani et al., Molecular cartography of the human skin surface in 3D, PNAS April 28, 2015; 112(17) E2120–E2129; Figure 4 courtesy of Prof. Theodore Alexandrov

DRIVING QUESTIONS

1 What is the human microbiome, and what is its role in health, disease, and forensics?

2 What are prokaryotes, and why are they classified into two distinct domains of life?

3 What are features of bacteria and archaea?

"Because microbes are ubiquitous, they can be tiny witnesses to the events of our lives."

—Jessica Metcalf

Jessica Metcalf holds up an image of a human hand. It's a ghostly blue, with yellow and green splotches glowing against a black background. The hand looks as if it's been dipped in fluorescent paint, then pressed onto film, leaving an eerie afterglow (see the chapter-opening photo).

She explains that the different colors correspond to different molecules found on the hand. One splotch might represent caffeine from a heavy coffee drinker, another might be a bit of antidepressant left on a fingertip, and a third might be the DNA from a particular type of bacterium.

Metcalf is a pioneer in the emerging field of microbial forensics. She seeks to use information from microbes to aid forensic investigations. The glowing hand comes from a colleague's project aimed at mapping the skin's chemical and microbial landscape.

"Because microbes are ubiquitous, they can be tiny witnesses to the events of our lives," says Metcalf, an evolutionary biologist at Colorado State University in Fort Collins. "Skin microbes hold great potential for forensic science." We each have our own unique collection of microbes living on and in us, and scientists hope to use these traveling companions as a kind of microbial fingerprint. Like traditional fingerprints, our microbial signatures are left on surfaces and may provide clues to an identity. But microbial traces can potentially provide even more data—information about a person's lifestyle, what that individual recently ate, whether the person wears makeup or takes certain medications.

Peter Bolter/Alamy

Computer keyboards and computer mice retain microbial fingerprints that can identify users.

Studies like the glowing hand project would have been impossible to do just a few years ago. But thanks to advances in DNA sequencing, it is now possible to make visible to the naked eye what previously required high-powered microscopes. What these new techniques have revealed is an entire world of microbes living among our own cells. Scientists call the community of microbes living in or in our bodies the human **microbiome.**

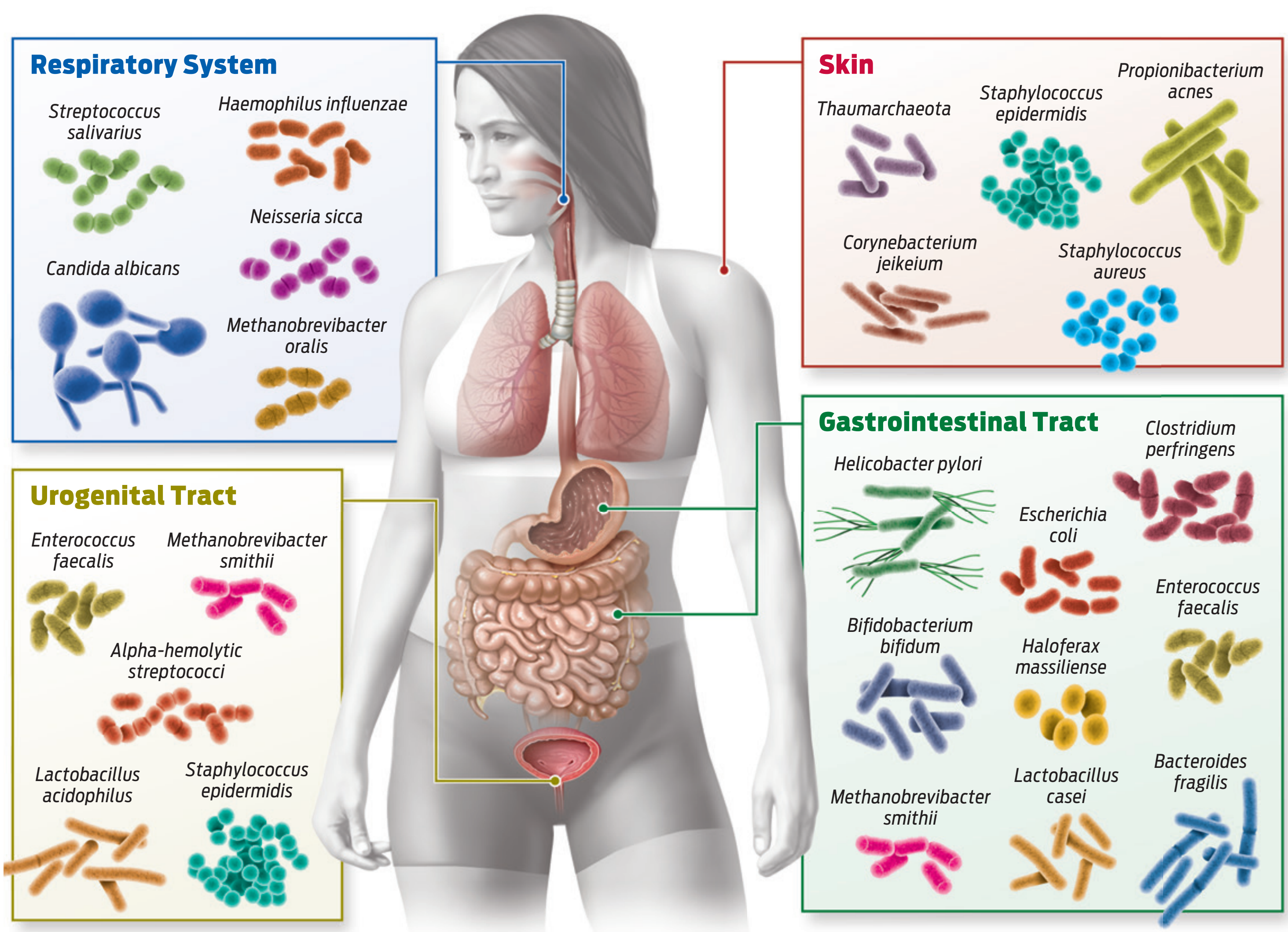
Our skin, our gut, and our respiratory and reproductive tracts are all home to diverse microbial organisms—among them, bacteria, archaea, protists, and fungi. These microscopic companions influence our susceptibility to disease and contribute to our normal physiology. By some estimates, there are at least as many microbial cells living on and in us as there are human cells making up our body **(INFOGRAPHIC 17.1)**.

MICROBIOME
A community of microbes at a particular location (e.g., on a person's skin or in the gut).

INFOGRAPHIC 17.1

The Human Microbiome

A huge diversity of microbes live on and in the human body. These resident microbes include prokaryotic bacteria and archaea, as well as eukaryotic fungi and protists. This community of microorganisms—the human microbiome—influences our health. While there are common organisms in the human microbiome, each person's individual microbiome is unique.



? **List three microorganisms likely to be found in a microbial fingerprint.**

The paper that many say launched the field of microbial forensics was published in 2010. Metcalf's former post-doctoral advisor, Rob Knight, showed that it was possible to identify individuals based on the bacterial profiles they left on computer keyboards and computer mice. Using this approach, he and his colleagues were able to correctly identify nine separate individuals based on the microbial fingerprints they left on these objects, even after the objects were left untouched for 2 weeks. Knight's lab is also responsible for the glowing human hand study.

The science of microbial forensics remains at the exploratory stage. So far, the only crimes microbes have helped solved have been on the television show *CSI: Miami.* Even so, many believe it is only a matter of time before our microbiomes become an important part of forensic science, complementing their increasing role in medicine and health.

Small But Mighty

▶ Properties of prokaryotic organisms

The vast majority of microbes making up the human microbiome are single-celled prokaryotic organisms—bacteria and archaea. Recall from Chapter 3 that **prokaryotes** have unique characteristics that distinguish them from eukaryotes. The DNA in a prokaryotic cell floats freely in the cytoplasm—rather than being housed in a nucleus, as in eukaryotes—and consists of a single, circular chromosome. (Some prokaryotes contain additional, small circular DNA molecules called plasmids that may confer an advantage in certain environments.) Prokaryotes are composed of a single cell that lacks internal membrane-bound organelles and is usually surrounded by a cell wall.

Prokaryotic organisms are tiny, on the order of 1–10 micrometers, which is about one-tenth the thickness of a human hair. Most are unicellular, but some are able to form multicellular colonies that can function as a single unit.

PROKARYOTE
A (typically) unicellular organism whose cell lacks internal membrane-bound organelles and whose DNA is not contained within a nucleus.

What prokaryotes lack in size, they make up for in numbers. Prokaryotes occupy virtually every niche on the planet, and most scientists agree that we have barely scratched the surface in cataloguing their numbers and diversity. There are more prokaryotic organisms in a handful of dirt than there are plants and animals in a rain forest. Each one of us carries trillions of these tiny organisms on and in our bodies.

There are several reasons why prokaryotes have been so phenomenally successful at the game of life. First, they reproduce quickly—sometimes dividing in less than an hour—and are thereby able to rapidly populate a given environment. They can shuffle and share DNA between their cells, an ability that provides them with abundant genetic variation on which natural selection can act. Their genetic differences also lead to differences in nutrition and metabolism, enabling them to survive on a wide range of food sources **(INFOGRAPHIC 17.2)**.

Interest in the microbiome has been growing exponentially since the 1990s, when techniques for identifying these invisible organisms became widely available. Many prokaryotic organisms are hard to grow in the lab, so they were difficult to study until newer techniques made it possible to analyze them in their natural habitats from their DNA alone.

Numerous projects are now under way to document the microbiomes of different environments: soil, hospitals, pets, even the International Space Station. But it's the forensic angle that has many researchers buzzing.

A Death Clock

▶ Microbial forensics

Metcalf first became interested in microbial forensics while studying DNA collected from ancient human remains. She and Rob Knight were interested in using this ancient DNA to reconstruct what the human gut microbiome was like before the modern invention of agriculture and antibiotics. Such information

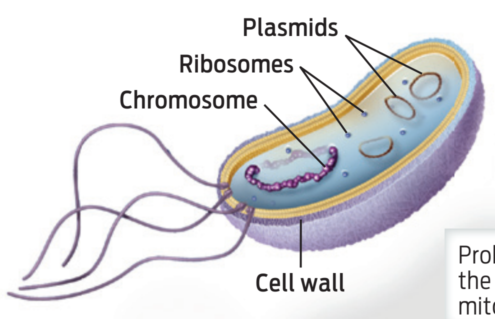
INFOGRAPHIC 17.2

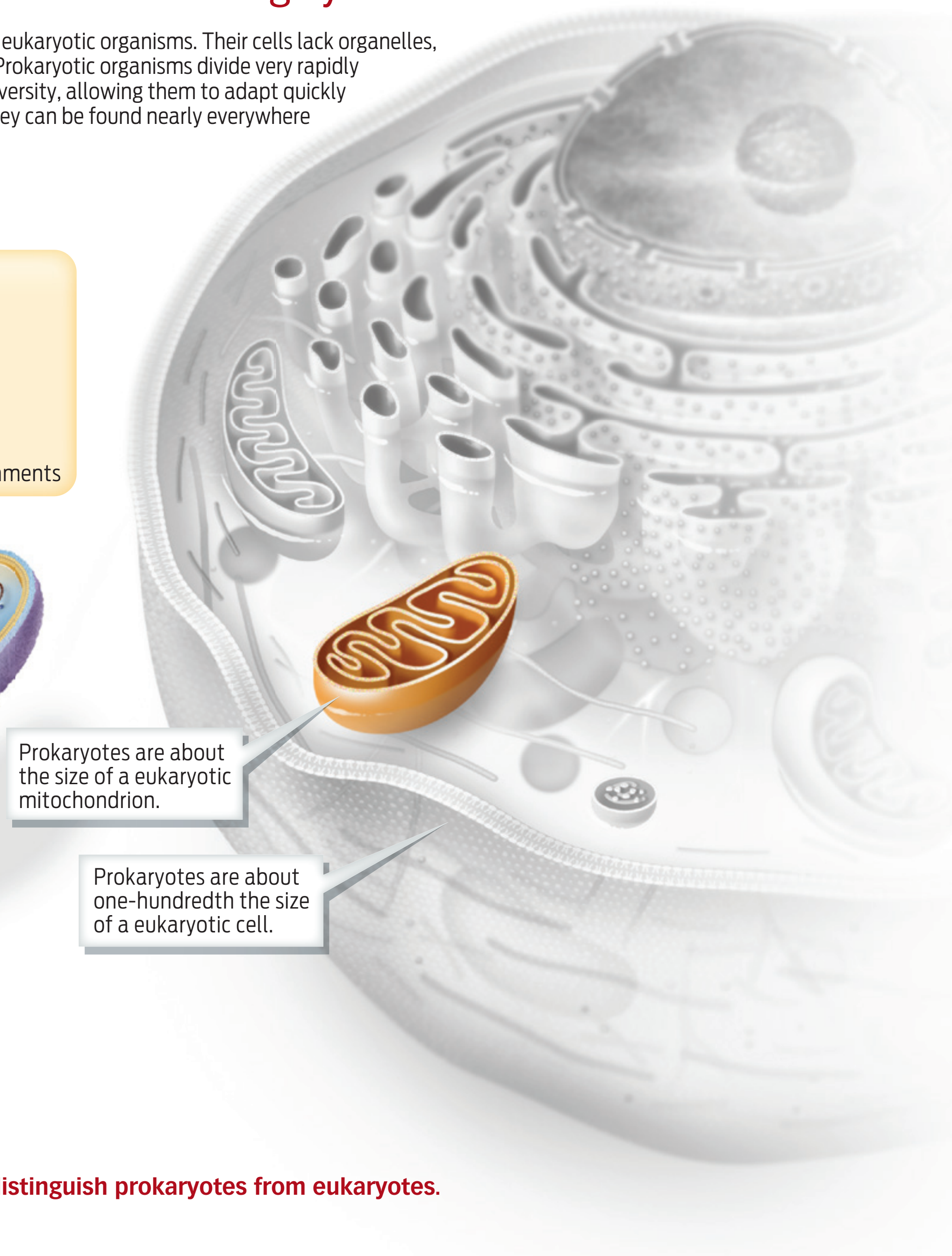
Prokaryotic Organisms Are Small but Highly Successful

Prokaryotic organisms are much smaller than eukaryotic organisms. Their cells lack organelles, and their DNA floats freely in the cytoplasm. Prokaryotic organisms divide very rapidly and can generate large amounts of genetic diversity, allowing them to adapt quickly to changing environments. Because of this, they can be found nearly everywhere on the planet.

Prokaryotic Organisms

- Typically single-celled
- No organelles
- DNA floats freely in cytoplasm
- Unique cell walls
- Divide and reproduce rapidly
- Adaptable to a variety of environments





? List at least three features that distinguish prokaryotes from eukaryotes.

could be useful for understanding our modern susceptibility to particular diseases. But the researchers quickly encountered a stumbling block.

"I started thinking about how, in order to interpret the microbial DNA from ancient samples, I needed to know how the microbiome changes during death and decomposition," Metcalf says.

When animals die, microbes from the environment begin to decompose their flesh. These fellow travelers in ancient remains would make interpreting microbiome DNA from these samples difficult.

And so began one of Metcalf's current projects: the quest to understand the microbiology of death. How does the microbiome change after we die? Which species become more common, and which less common? Are the common species the ones we carried with us in life, or do they come from the environment in which we are decomposing?

"I started thinking about how, in order to interpret the microbial DNA from ancient samples, I needed to know how the microbiome changes during death and decomposition."

—Jessica Metcalf

Metcalf began studying the microbiology of death in mice. She let mice decompose atop tiny soil graves. Each day, she or a lab member would swab parts of the animal corpses—head, torso, abdominal cavity—and the surrounding soil. Then she analyzed the DNA extracted from these samples to identify which microbes were most abundant.

By studying these changes in microbial life over time, Metcalf has discovered that most of the microbes that grow on us after death come from the environment, with a reduced contribution from our own microbiome. What's more, she found that the sequence of microorganisms that appear is largely consistent and predictable. In fact, she and her team could predict the time of death to within 3 days, even after 48 days of decay. She realized that these microbial changes would be used as a kind of "microbial clock," a term she coined.

Metcalf wondered, would the clock run the same way in humans? It's obviously much harder to come by dead humans than it is dead mice. That's why in recent years she's partnered with anthropological research stations, sometimes called (somewhat crudely) "body farms." At these facilities, cadavers that have been donated to science are left to decompose in a protected field. The research stations are used by various scientists and forensic professionals to understand the changes that occur after death.

Metcalf worked with scientists at the Southeast Texas Applied Forensic Science Facility to conduct the experiment. Four fresh human corpses were left to decompose in the field. At regular intervals, project scientists would swab the skin of the corpse as well as the surrounding soil.

As in mice, the microbial clock of decomposition in humans allowed the researchers to accurately establish the time of death: in this case, with an error range of 2–4 days over 25 days.

"There are clear forensics implications," Metcalf says of her research, which is funded in part by the Department of Justice **(INFOGRAPHIC 17.3)**.

Other active projects in the lab include studying how well skin microbes transfer to different common materials such as glass, plastic, metal, ceramic, and wood, and how long a person's skin microbial signature lasts after death.

Unseen Roommates

Our microbiomes may identify us, but they aren't glued to us. We continually shed a cloud of microbes as we move through the world, creating a kind of microbial aura. Metcalf recently turned her microbial detective skills to understanding this microbial aura in the context of people's homes.

Called the Home Microbiome Project, the study followed seven families, including their pets—for a total of 18 people, three dogs, and one cat—for 6 weeks. Study participants swabbed their hands, feet, and noses daily. They also swabbed surfaces in the house, like countertops, floors, light switches, and doorknobs.

"One thing that's become clear with microbiome research is that people have a unique individual skin microbiome that

INFOGRAPHIC 17.3

The Microbiology of Death

Five stages of decay take place when an animal dies. Each stage has predictable characteristics, including a predictable series of microbes that aid in the decomposition process. The presence of these characteristic microbes can be used as a microbial death clock to determine an organism's time of death.

Fresh

Shortly after death, blood pools in the lower parts of the body, turning tissue blue. Muscles turn rigid. Cells begin to break down from their own enzyme activity.

Bloat

Microbes within the body feed on body tissues. Anaerobic bacteria produce gases that give the body a bloated appearance. Gas pressure can cause skin to rupture.

Microbes at work:

Proteobacteria: *Pseudomonas*; Enterobacteriaceae
Proteobacteria: *Wohlfahrtiimonas; Ignatzschineria*
Basidiomycota: *Lysurus*

Active Decay

Ruptured tissues are exposed to oxygen. Aerobic microbes and insect larvae enter from the environment and feed on body tissue. Decaying tissues release fluids and strong odors.

Microbes at work:

Bacteroidetes: *Myroides*
Ascomycota: *Yarrowia*
Nematoda: Rhabditidae
Proteobacteria: *Psudomonadaceae*; Chromatiaceae; *Proteus*

New Zealand Geographic Issue 138, Mar-Apr 2016 "Game Over" by Dave Hansford

Advanced Decay

Insect larvae and microbes complete soft tissue decay and develop into beetles. Fluids from the body release carbon, nitrogen, and other nutrients to the surrounding soil.

Microbes at work:

Stramenopiles: *Plasmopara*
Zygomycota: *Mortierellaceae*
Firmicutes: *Planococcaceae*
Proteobacteria: *Acinetobacter*

Dry Remains

No soft tissue or fluid remains. Dry skin, cartilage, and bone are bleached white. Nutrients released to the surrounding soil result in increased plant growth.

Microbes at work:

Firmicutes: *Lactobacillales*
Firmicutes: *Sporosarcina*
Ascomycota: *Stromatonectria*

? **A decaying corpse has an abundance of Bacteriodes, Asomycoto, and Nematoda. At what stage of decay is this corpse?**

is somewhat consistent over time," Metcalf says. "You leave it in spaces that you occupy and you transfer it to objects that you touch."

The home study showed just how quickly we deposit our microbiome in the areas that we occupy. Within 24 hours of people moving into a house, their skin microbiomes blanketed surfaces. When a person left on a trip, that person's skin microbiome disappeared from the house within just a few days.

Picking up a phone, drinking from a glass, or even just walking through a room will leave behind millions of individual microbes from hundreds of species. These microbial fingerprints will then disappear rapidly from surfaces that are infrequently touched, but can persist for longer on frequently touched objects.

"Our classic fingerprints contain no temporal information in them," Metcalf says. "They could have been there for 10 seconds or they could have been there for 10 years. Whereas the microbial fingerprint has a temporal aspect to it because it will disappear over time."

Microbial fingerprints also contain information about our lifestyles. In another study, based on a staged home break-in, researchers were able to predict that one of the "burglars" had a least 10 alcoholic drinks a week and that the other was taking medicines for migraines. These clues came from the types of microbes that were left at the crime scene.

Revising the Tree of Life

▸ Prokaryotic domains of life

Microbes are invisible to the naked eye, and sometimes they even look similar under a microscope. So Metcalf and her colleagues need a different sort of vision to tell different species of microbes apart. That's where DNA comes in handy. Because DNA is common to all life, yet its exact sequence changes over time as organisms evolve, it can be used to identify individual species. DNA sequences that are unique to each species serve as a kind of identifying barcode.

From a sample obtained via a swab, researchers can isolate DNA, amplify it using polymerase chain reaction (PCR) technology (Chapter 7), and then sequence it using automated sequencing techniques than can run thousands of samples at a time.

The methods that Metcalf and her colleagues use to study the microbiome are extensions of an approach pioneered in the 1970s by Carl Woese, an American biologist. Woese was interested in improving scientists' understanding of the evolutionary history of life. He wanted to base his tree of life not on differences in appearance or behavior among organisms, but on actual degrees of genetic relatedness.

At the time, no genomes had been sequenced, and large-scale sequencing of DNA and RNA was still in its infancy. Thus, Woese had to overcome technical as well as conceptual hurdles. His first conceptual hurdle was to identify a molecule that is common to all life, but whose sequence changes slowly over time. He settled on the 16S ribosomal RNA. This RNA molecule makes up one subunit of the prokaryotic ribosome, the molecular machine that manufactures proteins. One part of this molecule is almost identical across all prokaryotic organisms, while another part is more variable. These tiny variations in the sequence of the ribosomal RNA molecule, Woese realized, could be used to identify individual species of bacteria and measure their relatedness. Bacterial species with more similar 16S RNA sequences could be placed closer together on the tree, indicating their closer relationship.

All went well until Woese got to a group of microbes known as methanogens. These curious creatures don't use oxygen to live; in fact, oxygen is toxic to them, so great care must be used to grow them in the lab. Instead of carbon dioxide, these microbes release methane into the atmosphere (methanogen means "methane-generator"). They grow in low-oxygen environments like wetlands, as well as inside the guts of certain animals.

When Woese sequenced the methanogens' 16S RNA, he discovered that it looked nothing like the bacterial sequences he had collected. This finding bewildered Woese at first. But over time he came to what he thought was the inescapable conclusion: methanogens were not bacteria at all. They represented another domain of life, distinct from both bacteria and eukaryotes. He called this group archaea, from the Greek word for "ancient" **(INFOGRAPHIC 17.4)**.

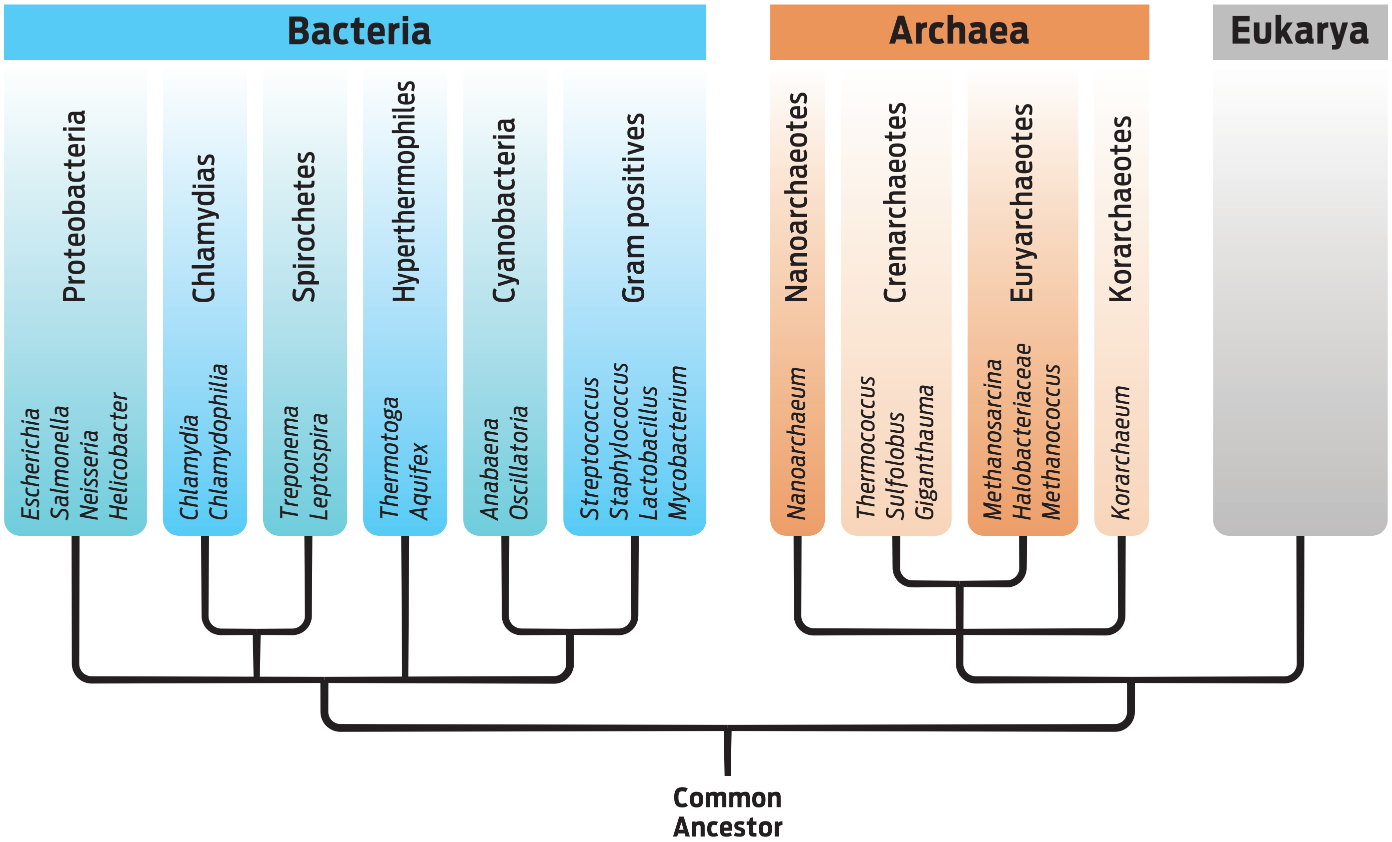
Woese's proposal of a new domain of life did not immediately catch on with other biologists. In fact, many were skeptical of the idea at first. But over time, as more evidence accumulated, they were forced to admit that Woese was right. The familiar tree of life that many scientists had grown up with, featuring five kingdoms with a single prokaryotic kingdom called "Monera," needed to be overhauled (see Chapter 16).

In Woese's day, the process of sequencing a single 16 RNA molecule was painstaking and could take the better part of a year. Today, thanks to advances in technology,

INFOGRAPHIC 17.4

Bacteria and Archaea, Life's Prokaryotic Domains

All living organisms fall into one of the three domains of life. Within each domain, there are subgroups of organisms, grouped together based on their evolutionary relationships, as determined by 16S rRNA sequences. Two of the domains of life, Bacteria and Archaea, have organisms with prokaryotic cells, but each has a distinct evolutionary history. Archaea are more closely related to eukaryotes than to bacteria.



? What is the basis for the classification of bacteria and archaea into separate domains, despite their common prokaryote structure?

it's much easier and faster. But the premise of using genetic information to identify microbes is much the same, except that instead of using the 16S RNA molecule itself, scientists now use 16S RNA gene sequences to infer the presence and relative abundance of different microbial species. This technique has allowed scientists to learn more about the abundance and identities of prokaryotic organisms in different locations, from soil to glaciers to coral reefs **(INFOGRAPHIC 17.5).**

Lifestyles of the Small and Infamous

▶ Bacterial structure and diversity

Much of the human microbiome is made up of **bacteria,** the unsung workhorses of the planet. These tiny organisms include commonly encountered microbes such as the *Escherichia coli* present in our gut and the *Staphylococcus aureus* colonizing our skin, as well as not so commonly encountered ones like the fluorescence-emitting bacteria living in partnership with a sea squid.

BACTERIA
One of the two domains of prokaryotic life; the other is Archaea.

While all bacteria are prokaryotic, and most possess a cell wall, their genetic diversity translates into a wide variety of differences in nutrition, metabolism, shape, and lifestyle. Bacteria live just about everywhere on the planet, from briny lakes submerged under miles of ice, to hazardous waste disposal sites, and everything in between.

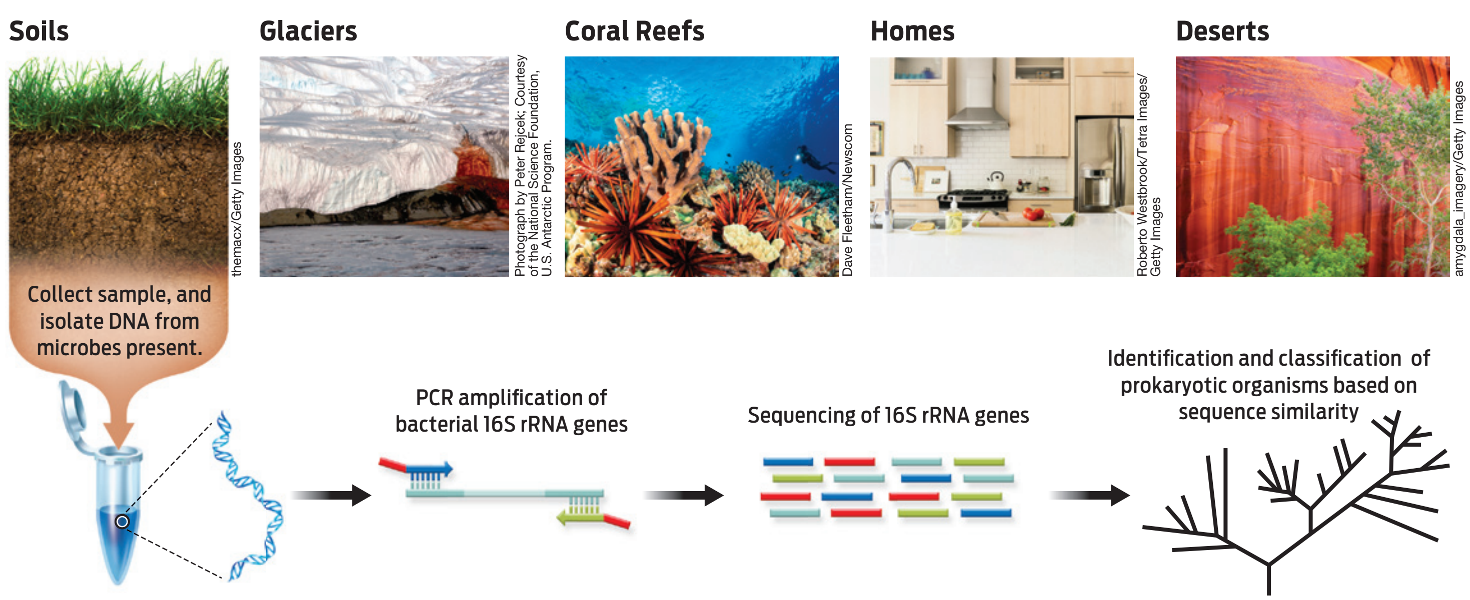
Like all organisms, bacteria can be categorized by what they eat. Some bacteria are autotrophs (literally, "self-feeders"): they are able to make their own food directly, using material from the nonliving environment, ranging from carbon dioxide to rocks. Others are heterotrophs (literally, "other feeders"): they must consume material from other living organisms to obtain food.

One of the largest and most important groups of autotrophic bacteria is the cyanobacteria. These bacteria are found in oceans and freshwater, as well as on exposed rocks and soil—virtually everywhere sunlight can

INFOGRAPHIC 17.5

Discovering the Hidden Worlds of Microbes

Distinct collections of prokaryotic organisms are being discovered and characterized in a variety of different environments. 16S rRNA genes are used to identify individual members of a specific microbial community.



? **Which sequence is used to identify members of a given microbiome?**

reach. Cyanobacteria use the energy of sunlight to carry out photosynthesis in a manner similar to plants: they take in CO_2 to generate carbohydrates (food) and in the process produce much of the oxygen that other organisms, including humans, rely on.

Heterotrophic bacteria include those that obtain food by consuming material from living or dead organisms. Such bacteria play an important role in decomposition: they feed on dead organisms, allowing carbon and other elements—which would otherwise be trapped in dead organisms, sewage, or landfills—to be recycled. They are also useful in bioremediation projects. For example, some types of bacteria break down droplets of oil, much the same way that humans digest butter, so they help clean up oil spills naturally.

Bacteria break down their food molecules through a variety of metabolic pathways—some of which require oxygen, and some of which do not. For example, many bacteria employ the anaerobic process of fermentation (see Chapter 6) to get energy from food. The products of fermentation can be valuable (and tasty) to humans. You may have seen "L. bulgaricus" listed as an ingredient of yogurt. Live *Lactobacillus bulgaricus* bacteria are present and hard at work in the yogurt, fermenting sugars into lactic acid. This acid helps the milk solidify into yogurt and gives yogurt its tangy taste. Other bacteria use oxygen to break down organic molecules, like the aerobic bacteria that feast on an oil spill.

In addition to nutritional and metabolic differences, bacteria display a variety of structural adaptations that suit their various lifestyles. They come in different shapes: spherical (in which case they are known as cocci), rod-shaped (bacilli), and spiral (spirochetes). Many bacteria are equipped with **flagella,** tail like structures that project from the cell and rotate like propellers to help it move. For example, the bacterium *Helicobacter pylori,* the most common cause of stomach ulcers, uses its flagella to propel itself through the gastric mucus found in the stomach. **Pili** are shorter, hairlike appendages that enable bacteria to adhere to a surface. *Neisseria gonorrhoeae,* the bacterium that causes the sexually transmitted disease (STD) known as gonorrhea, uses its pili to remain attached to the lining of the urinary tract. Without pili, the bacteria would be flushed out by the flow of urine.


James Peake/Alamy

Hawaiian bobtail squid (*Euprymna scalopes*) live in symbiosis with fluorescent bacteria that reside in the squid's light organ. The illumination from the bacteria helps the squid avoid casting a shadow on a moonlit night, making it less noticeable to prey.

Other bacteria are surrounded by a **capsule,** a sticky outer layer that helps the cell adhere to surfaces and to avoid the defenses of the host. *Streptococcus mutans,* for example, produces a capsule that allows it to adhere to teeth, where it forms the plaque that can lead to cavities.

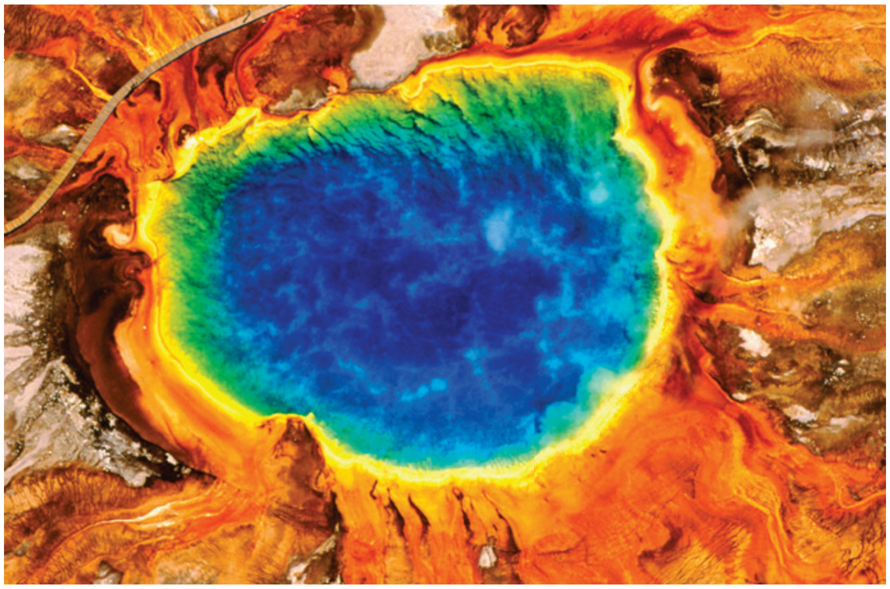
The highly resourceful bacteria have forged a diverse range of living arrangements with other creatures. Many live in close association, or **symbiosis,** with other organisms—often to the benefit of one or both partners. Lactobacilli reside naturally in the female vaginal tract, for example. There they feed on naturally occurring sugars, which they ferment to lactic acid. The resulting acidity of the vaginal tract suppresses the growth of yeast, preventing yeast infections. Antibiotics

FLAGELLA (SINGULAR: FLAGELLUM)
In bacteria, long, slender appendages extending from some bacterial cells, used in movement of the cell.

PILI (SINGULAR: PILUS)
Short, hairlike appendages extending from the surface of some bacteria that enable them to adhere to surfaces.

CAPSULE
A sticky coating surrounding some bacterial cells that adheres to surfaces.

SYMBIOSIS
A relationship in which two different organisms live together, often interdependently.



Charles O'Rear/Getty Images

Yellowstone's Grand Prismatic Hot Spring. Heat-tolerant bacteria and archaea live in this 80°–100°C, low-pH, high-sulfur aquatic environment that bubbles up from below Earth's crust. The rainbow colors that ring this spring come from light reflecting off the pigments made by different microbe species.

taken for a bacterial infection are likely to kill the resident lactobacilli as well as the invaders, and a yeast infection may be an unhappy side effect.

Another example of beneficial bacterial symbiosis is provided by *Vibrio fischeri.* This bioluminescent bacterium lives and feeds inside the light organs of certain species of squid. The glow-in-the-dark *Vibrio* produces light beneath the squid. That light helps obscure the shadow that the squid might cast on a moonlit night, making it less noticeable to its prey as it hunts.

Unfortunately, not all bacteria are beneficial to the host. While the vast majority of bacteria do not cause human disease, some do. Organisms that cause disease are called **pathogens.** Many pathogenic bacteria cause disease by producing toxins that harm their hosts. For example, certain strains of *E. coli* secrete a potent toxin that causes bloody diarrhea and sometimes even kidney failure and death in its host. Washing hands before preparing or eating food will help prevent transfer of *E. coli* from dirty hands, and cooking meat to recommended temperatures will kill contaminating bacteria, reducing the risk of toxin-producing *E. coli* entering the body with a meal.

Not all pathogens produce toxins. Some cause disease by living and reproducing in the body and interfering with its normal processes. An example is the bacterium *Treponema pallidum,* which causes syphilis, an STD.

In some cases, bacteria grow as a biofilm, a collection of bacteria that adhere to a surface and to one another. Bacteria in biofilms secrete sticky substances that encase the biofilm, protecting the bacteria. Up to 65% of all bacterial infections may be associated with biofilms.

Sometimes the line between harmless and harmful bacteria can be blurred. Organisms that can—but don't always—cause disease are known as opportunistic pathogens. For example, most of us have *S. aureus* on our skin at many times during our lives. Most of the time, this bacterium does not cause any harm, but if it penetrates the skin—through a wound, for example—it can cause a serious infection and even death, as discussed in Chapter 13 **(INFOGRAPHIC 17.6)**.

The bacterial members of the human microbiome are not distributed evenly across the body. Regions of the body such as the face, chest, and back that contain abundant oil-producing glands tend to harbor fat-loving microorganisms. In contrast, less exposed regions, such as the groin, armpit, and toes, tend to harbor species that prefer a warm and wet environment.

Some Like It Hot

▸ Archaeal structure and diversity

Archaea are similar to bacteria in that archaea are single-celled organisms that lack a nucleus, but genetically they are as different from bacteria as humans are. All those genetic differences add up to a number of unique features that distinguish archaea from bacteria. For example, whereas bacteria

PATHOGEN
A disease-causing agent or organism.

ARCHAEA
One of the two domains of prokaryotic life; the other is Bacteria.

have cell walls made of the molecule peptidoglycan, archaea have cell walls composed of other molecules. Archaeal cell membranes can also have a different chemical composition, and archaea rely on unique forms of metabolism.

Though archaea are found in many run-of-the-mill habitats such as rice paddies, forest soils, lake sediments, and our own respiratory tract, the most well-known species are the so-called extremophiles, found in extreme environments. Many of these extreme-loving archaea are hyperthermophiles—organisms that can survive only at extremely high temperatures. Many hyperthermophilic archaea are anaerobic and rely on sulfur instead of oxygen in capturing energy from food. Sulfur-rich hot springs like those in Yellowstone National Park are home to these archaea.

Some archaea are halophiles, or "salt lovers." These archaea prefer a home saturated in salt, which would shrivel most other living things. Their presence is detectable by the

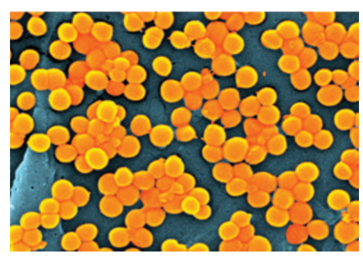
INFOGRAPHIC 17.6

Exploring Bacterial Diversity

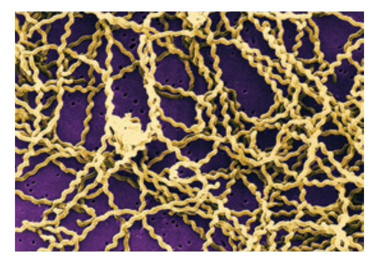
Bacteria live in every imaginable place on Earth and have a diverse array of lifestyles. Their unique structural and metabolic adaptations have enabled them to become a dominant force of life on the planet.


STEVE GSCHMEISSNER/SCIENCE PHOTO LIBRARY/Getty Images

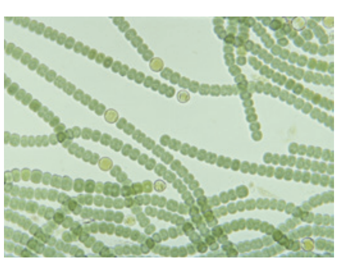
Rod-shaped
Lactobacillus


Janice Carr/Getty Images

Sphere-shaped
Staphylococcus


Callista Images/AGE Fotostock

Spiral-shaped
Leptospira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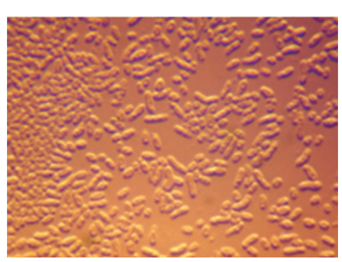
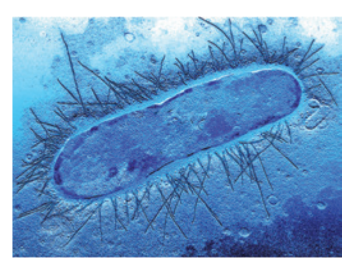
Photosynthetic
Anabaena cyanobacteria


Michael Abbey/Science Source

Eats oil
Ochrobactrum anthropi


BSIP/Getty Images

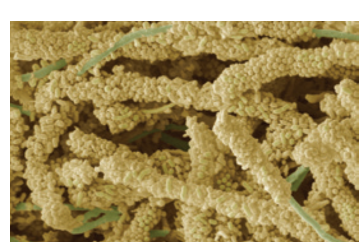
Pathogenic
Escherichia coli


CDC/JANICE CARR/AGE Fotostock

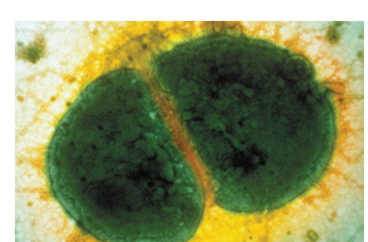
Thrives in a high-salt environment
Halomonas elongata


BSIP/UIG/Getty Images

Flagella
Helicobacter pylori


STEVE GSCHMEISSNER/SPL/Getty Images

Capsule
Streptococcus mutans


Kwangshin Kim/Getty Images

Pili
Neisseria gonorrhoeae

? Flagella, capsules, and pili can all be found on pathogenic bacteria. Pick one of these structures, and describe how it might contribute to the development of disease.

aerialarchives/Alamy/AGE Fotostock

Aerial view of salt ponds in San Francisco Bay.

colorful pigments they produce—bright reds, yellows, and purples—as seen in salt ponds in San Francisco Bay **(INFOGRAPHIC 17.7)**.

Archaea play important roles in the lives of many animals, most especially those with a rumen, a compartment in the digestive tract. Methane-producing archaea in the rumen help these animals—which include cattle, sheep, goats, deer, and giraffes—with the digestion of cellulose-rich grasses. The methane that cows produce is actually a significant contributor to rising greenhouse gases (see Chapter 23).

Some scientists speculate that the specific archaea present in our guts may account for individual differences in the types (and smells) of gases we emit—for example, as flatulence.

Mother's Milk

▸ Acquiring the microbiome

Where do we get our unique microbiome? We start acquiring it during the birth process. When a baby passes through the birth canal of its mother, it acquires a collection of microbes from her vaginal tract. [Babies born by cesarean section (C-section) do not pick up these bacteria in this way, but can be provided with them after birth.] Breastfeeding further contributes to the human microbiome. Children are essentially fully colonized by 1–2 years of age. Genetics, what we eat, and the medicines we take also contribute to differences in our microbiomes.

Metcalf is interested in these differences as well. She has studied the gut microbiome of people eating traditional diets, such as the Yanomami who live in remote regions of the Amazon rain forest. The goal of this research is to understand how the human gut microbiome has changed over time, from before industrialization until now.

One of the most significant changes in that time, of course, was the development of antibiotics (Chapter 3). We also eat differently now. These changes have undoubtedly altered the composition of our gut microbiome, but exactly how remains an open question.

Metcalf and her colleagues have found that the gut microbiomes of people eating modern, Westernized diets are much less diverse than the gut microbiomes of people who eat more traditional diets. Most notably, the latter have more *Treponema* bacteria, many of which can cause disease.

Having fewer potential pathogens in our guts might sound like a good thing, and for the most part it is. We now suffer from many fewer infectious diseases as a result of improvements in our hygiene practices, such as having access to clean water and sewage systems.

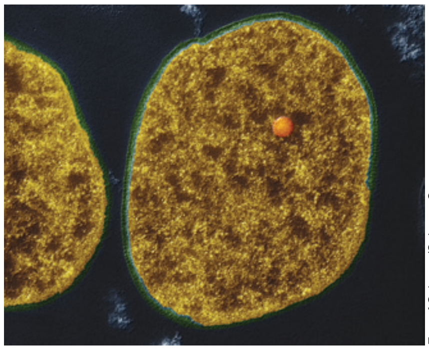
But Western lifestyles are also associated with increases in other types of diseases that typically do not affect members of traditional societies. Heart disease is one such example, but more mysterious illnesses like autoimmune diseases and autism are more prevalent in Westernized societies as well.

Some scientists hypothesize that these diseases may have a connection to the modern microbiome. The idea is this: as cultures around the world become more "Western"

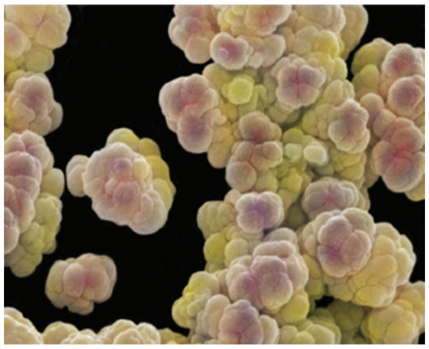
INFOGRAPHIC 17.7

Exploring Archaeal Diversity

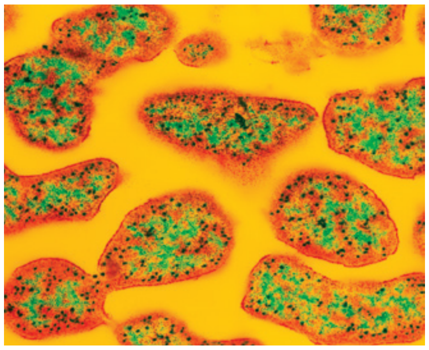
Archaea are sometimes known as "extremophiles." They live in diverse environments, often surviving and thriving in very harsh conditions.



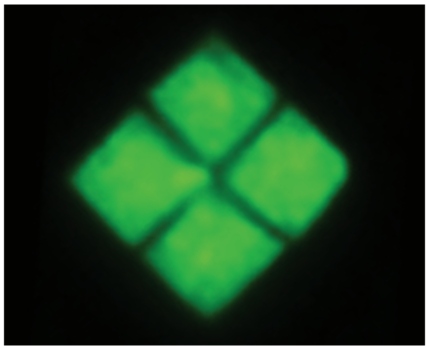
Sulfolobus
Considered extremophiles because they can grow at 70ºC and at pH 2.0



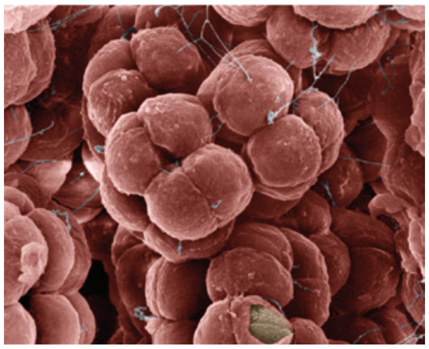
Methanosarcina
Has unusual cell walls and membranes, and produces methane



Methanococcoides
Grows in cold temperatures as low as −2.5ºC



Haloquadratum walsbyi
Thrives in water temperatures well above boiling, at least 120ºC



Halobacteriales
Not only tolerates but requires a high-salt environment to live

? What are three extreme conditions in which some archaea can survive?

in terms of having access to antibiotics and safe drinking water, the diversity of their gut bacteria declines—as does the incidence of infectious disease. At the same time, we start having higher incidences of chronic illnesses connected to the immune system, such as allergies, Crohn's disease, autoimmune disorders, and multiple sclerosis. This correlation between low diversity of gut bacteria and higher risk of diseases related to the immune system has led to hypotheses about the role of a diverse microbiome in health and disease.

The possibility of a connection between a diverse microbiome and health has led to treatment approaches—none validated as of yet—that involve introducing these organisms back into the gut of affected individuals. The hope is that they will help reset the immune system and ease symptoms.

An additional remarkable finding from Metcalf's research is that genes for antibiotic resistance are found in microbes from indigenous peoples who have never encountered antibiotics. This evidence suggests that wild populations of bacteria already had these genes in their gene pool. The presence of these genes helps account for the rapid emergence of antibiotic resistance among many different bacterial groups today (see Chapter 13).

Microbiome Hope and Hype

Health applications of the microbiome

People are understandably excited about the microbiome and its implications for health. The trillions of tiny organisms that share our bodies with us cannot help but shape our existence. An increasing body of evidence shows that having a less diverse microbiome can increase the risk of harmful conditions such as allergies, asthma, obesity, and type 1 diabetes.

Preparing a fecal transplant.

One of the clearest examples of the microbiome playing a role in health is the value of fecal transplants in curing people of dangerous gut infections. *Clostridium difficile,* or *C. diff* for short, causes a dangerous infection that produces intense diarrhea and inflammation of the colon; it can be fatal. People often acquire a *C. diff* infection after taking antibiotics while in the hospital. The antibiotics kill off pathogens and beneficial bacteria, but not *C. diff,* which can then gain a foothold in the body and grow out of control.

So far, the only treatment that has worked consistently to cure *C. diff* is a fecal transplant—transferring stool from a healthy person to the gut of the sick person. The procedure, while unappealing to some, is effective between 80% and 90% of the time.

Some evidence links the microbiome to the growing obesity crisis in industrialized countries. Scientists have shown that they can make a mouse obese by transferring the gut microbiome of an obese human to it using a fecal transplant. And numerous studies have shown that the gut microbiome of obese people is often different from that of non-obese people. Whether this is a cause or an effect of obesity is not yet clear.

Similar results have been found using mice engineered to display traits of autism. It's possible to alter a mouse's display of autism symptoms by altering its gut microbiome, leading some scientists to propose a kind of gut–brain axis.

But as microbiome expert Elisabeth Bik points out, it can very hard to rule out other explanations. According to Bik, "With autism specifically, there's a big confounding factor, which is that kids with autism are often very picky about food and have idiosyncratic diets. So that could be influencing their gut microbiome."

"There are clear hints that obesity might be in part due to the microbiome and might be transferable through the microbiome," she says. "But a lot of these experiments are done in mice that have a tendency to become obese. So these mice will already become obese very easily."

In many cases, people's excitement about the microbiome has outstripped the science, Bik suggests. Most of the relationships that have been observed are correlations, which of course do not always imply causation (Chapter 1). This has led, unfortunately, to an abundance of hype in the news.

Hype aside, can we alter our microbiome intentionally? Perhaps the best and easiest thing a person can do to ensure a healthy gut, Bik says, is to regularly consume fiber, which can be found in many fruits and vegetables, as well as in legumes, whole grains, and nuts. Many of our "good" bacteria thrive on fiber, which they help us digest, and in the process release helpful chemicals such as certain short-chain fatty acids. These molecules both strengthen the mucus layer of the gut lining and reduce gut inflammation. A thriving population of beneficial bacteria nourished on fiber can prevent the growth of pathogenic bacteria. If not kept in check, such pathogenic bacteria will explode in numbers and destroy the mucus layer that protects intestinal cells.

Bacteria in the gut can also use the nutrients from fiber to synthesize vitamins, such as vitamin K. These vitamins contribute to human health, possibly preventing vitamin deficiencies. For example, people on a diet deficient in vitamin K did not develop a vitamin K deficiency, but people who had been given antibiotics to reduce their gut microbes developed a vitamin K deficiency while on the same vitamin K–restricted diet. Fiber is an example of a **prebiotic,** a component in food that encourages the growth of beneficial gut microbes **(INFOGRAPHIC 17.8).**

Another common—if less scientifically supported—method of attempting to alter one's gut microbiome is to consume **probiotics,** which are edible concoctions of living microorganisms. Examples of

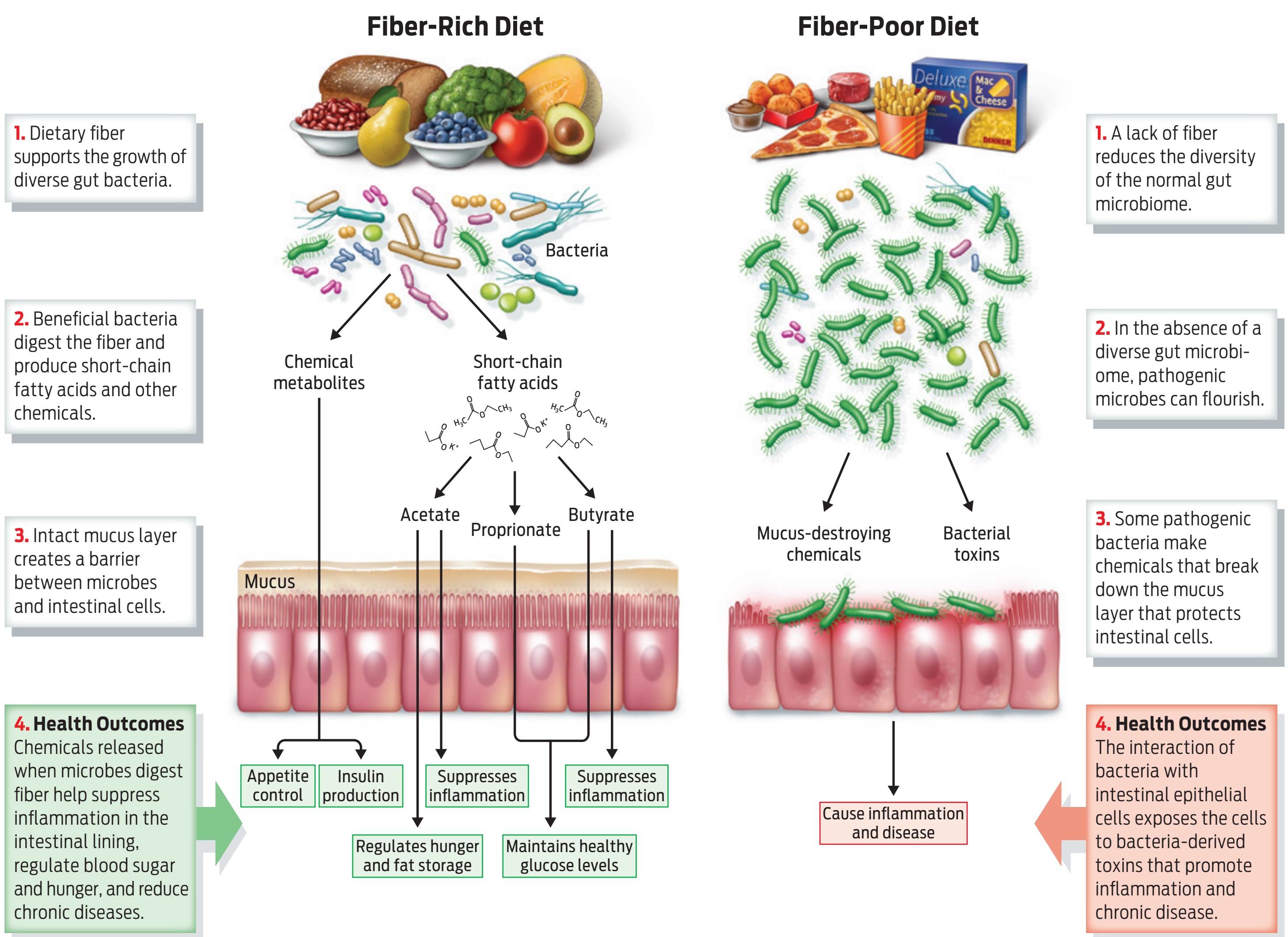
PREBIOTICS
Components of food (like fiber) that promote the growth of beneficial microbes.

PROBIOTICS
Living bacteria intended to be consumed and introduced into the microbiome (e.g., yogurt or capsules containing bacteria).

INFOGRAPHIC 17.8

A Healthy Gut Microbiome Defends Against Chronic Disease

Beneficial gut microbes flourish on a high-fiber diet and compete with pathogenic bacteria that can cause illness. They help maintain a thick mucus layer to protect epithelial cells from microbial damage. In addition, they use fiber as a source of nutrition and produce vitamins and chemicals that contribute to gut and overall health.



? From a microbiome perspective, why is a high-fiber diet important to health?

> *"There are clear hints that obesity might be in part due to the microbiome and might be transferable through the microbiome."*
>
> **—Elisabeth Bik**

commonly consumed probiotics include yogurt with "live cultures" and kefir, as well as capsules containing specific living microorganisms. Although the sale of such products is big business, very few probiotics have been shown to alter the human microbiome in any consistent or lasting way. While the bacterial strains contained in such products are easy to grow and manufacture, they aren't especially well adapted to growing inside the human gut. They can't compete with our normal gut microbiome for nutrients and end up moving right through us. As the science writer Ed Yong notes in his book *I Contain Multitudes* (2016), probiotic treatments are "like a breeze that blows between two open windows": they don't stick around long enough to make much of a difference.

So far, the strongest evidence in support of probiotics has come from their use in treating a very specific, life-threatening condition affecting the gut of preterm infants. In this case, the strains used are carefully chosen and grown for this particular purpose.

Bik isn't fazed by the many questions that remain about the microbiome and its role in health. She points to the Human Genome Project as a guidepost.

"Everybody thought, 'Okay, if we sequence the human genome, we'll be able to cure any disease.' That didn't happen. But it has led to a whole new field, personalized genomics. There's 23andMe and similar tests. There's a lot of new health information now.

"So there's a lot of hope, but science is much slower than most people think it is. Fifteen years ago, we had no idea what bacteria were living in a healthy gut. Now we do."

Metcalf, too, remains optimistic. She's currently working with the American Academy of Forensic Sciences to figure out how her work on the microbial clock could aid forensic researchers.

"We're trying to bring these methods out of the research lab and into practice," she says. ■

CHAPTER 17 Summary

Driving Question 1 What is the human microbiome, and what is its role in health, disease, and forensics?

- The human microbiome consists of the microorganisms—bacteria, archaea, protists, and fungi—living on and in us.
- Our microbiome begins to be acquired at birth.
- Our individual microbiome can be used to identify objects that we have touched, based on the microbial fingerprints that we leave.
- Disruptions to our microbiome can lead to disease.
- Restoration of our microbiome (e.g., through fecal transplant) can restore a state of health.

Driving Question 2 What are prokaryotes, and why are they classified into two distinct domains of life?

- Prokaryotes are unicellular organisms that lack internal organelles and whose DNA is not contained in a nucleus.
- Prokaryotes are found in virtually every environment on Earth, even those with seemingly inhospitable conditions.
- Genetic analysis has led to the categorization of life into three domains: Bacteria, Archaea, and Eukarya. Each domain of life has a distinct evolutionary history.
- Both bacteria and archaea have prokaryotic cells, but they otherwise differ in their genetics, biochemistry, and lifestyles.

Driving Question 3 **What are features of bacteria and archaea?**

- Bacteria are a diverse group of prokaryotic organisms with many unique adaptations, such as flagella and capsules, that allow them to live and thrive in many environments.
- Some bacteria are disease-causing pathogens, but most are harmless and many are even beneficial. Cyanobacteria, for example, are responsible for much of the photosynthesis that supports life on Earth.
- Often known as "extremophiles," archaea live in some of the most inhospitable conditions on Earth. Many archaea flourish in less extreme environments as well.

More to Explore

- Solving Crimes with the Necrobiome: https://www.youtube.com/watch?v=B_lHQSxz9GI
- Microbiomania: https://phylogenomics.blogspot.com/p/blog-page.html
- Microbiome Digest — Bik's picks https://microbiomedigest.com/
- Metcalf, J. L., Xu, Z. Z., Bouslimani, A., Dorrestein, P., Carter, D. O., and Knight, R. (2017). Microbiome tools for forensic science. *Trends Biotech* 35(9):814–823.
- Metcalf, J. L., Carter, D. O., and Knight, R. (2016). Microbiology of death. *Curr Biol* 26(13):R561–R563.
- Yong, E. (2016). *I Contain Multitudes: The Microbes Within Us and a Grander View of Life*. New York: Ecco.

CHAPTER 17 Test Your Knowledge

Driving Question 1 **What is the human microbiome, and what is its role in health, disease and forensics?**

By answering the questions below and studying Infographics 17.1, 17.3, and 17.8, you should be able to generate an answer for this broader Driving Question.

Know It

1. Where are members of the human microbiome found?
- **a.** skin
- **b.** gut
- **c.** feces
- **d.** respiratory tract
- **e.** all of the above

2. Most members of the human microbiome are prokaryotic. What does this mean?
- **a.** Their cells are very similar to human cells.
- **b.** They lack a nucleus, but contain other membrane-bound organelles.
- **c.** They lack all membrane-bound organelles.
- **d.** Their DNA is not contained in a nucleus.
- **e.** both c and d

3. How is the human microbiome acquired?

Use It

4. One baby is born by C-section and is formula-fed for its first year of life. Another baby is born by vaginal delivery and is breastfed for its first year of life. What differences, if any, might you expect in the microbiome of these two children at 1 year of age? Explain your answer.

5. People who are eating a "Western" diet have their gut microbiome sampled through a fecal sample. Half of the people then adopt a high-fiber, plant-based diet (similar to many traditional diets throughout the world). What do you predict about the gut microbiome in each group? If the "traditional" diet is followed for decades, what do you predict about the incidence of autoimmune diseases? How much confidence would you have in the results of such a study (in which you ask a group of people to follow a new diet for decades)?

Driving Question 2 **What are prokaryotes, and why are they classified into two distinct domains of life?**

By answering the questions below and studying Infographics 17.2, 17.4, and 17.5, you should be able to generate an answer for this broader Driving Question.

Know It

6. Organisms are placed into one or another of the three domains of life on the basis of
- **a.** cell type.
- **b.** physical appearance.
- **c.** evolutionary history as assessed by genetic relatedness.
- **d.** ability to cause disease.
- **e.** degree of sophistication—that is, how evolutionarily advanced they are.

7. Which domain(s) of life contain organisms with a prokaryotic cell structure?
- **a.** Archaea
- **b.** Bacteria
- **c.** Eukarya
- **d.** Archaea and Bacteria
- **e.** Archaea, Bacteria, and Eukarya

8. The absence of membrane-bound organelles in a cell tells you that the cell must be

- **a.** from a member of the domain Bacteria.
- **b.** from a member of the domain Archaea.
- **c.** from a member of the domain Eukarya.
- **d.** either a or b
- **e.** either b or c

9. The term *prokaryotic* refers to

- **a.** a type of cell structure.
- **b.** a domain of life.
- **c.** a group with a shared evolutionary history.
- **d.** a type of bacterium.
- **e.** a type of archaea.

10. What did Carl Woese use to group organisms into three distinct domains?

- **a.** the presence or absence of a nucleus
- **b.** the sequence of an rRNA molecule
- **c.** the ability to survive and reproduce in extreme environments
- **d.** the presence or absence of a cell wall

Use It

11. Why were bacteria and archaea originally grouped together?

12. When first discovered, archaea were called "archaebacteria." Why do you suppose this name was used? What are the strengths and weaknesses of this earlier term?

13. Can you use cell structure to classify a cell as either bacterial or archaeal? Explain your answer.

14. Many prokaryotic organisms can carry out photosynthesis. How is this beneficial to humans?

Driving Question 3 What are features of bacteria and archaea?

By answering the questions below and studying Infographics 17.6 and 17.7, you should be able to generate an answer for this broader Driving Question.

Know It

15. If you were looking for a bacterium, where would expect to find one?

- **a.** on your skin
- **b.** in soil
- **c.** in the ocean
- **d.** associated with plants
- **e.** any of the above

16. When examining archaea, which of the following is *not* a trait that you could expect to find?

- **a.** ability to grow at high temperatures
- **b.** a nucleus
- **c.** ability to grow at extremely acidic pH
- **d.** a cell wall
- **e.** ability to survive in a high-salt environment

Use It

17. If you are unable to culture archaea from an environmental sample, is it safe to conclude that there are no archaea present? Why or why not?

18. Halophilic (salt-loving) archaea are able to prevent osmotic water loss from their cells, even in high-salt environments. What is one mechanism by which they could prevent water loss and thrive in high-salt environments? Hint: Think about osmosis and the salt concentration in the environment versus inside their cells.

19. If *Neisseria gonorrhoeae* had no pili, would it still be a successful pathogen? Explain your answer.

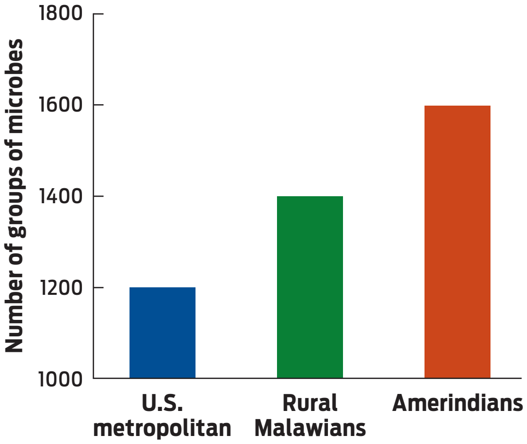
Interpreting Data

Apply Your Knowledge

20. It has been suggested that increasing rates of immune disorders, such as asthma and some food allergies, are the result of a reduced exposure to a diversity of "friendly" bacteria in the human microbiome.

- **a.** Based on the data shown in the bar chart, which population would you predict to have the lowest rates of asthma and allergies? Note that the Amerindians are from rural communities in Venezuela.
- **b.** If your prediction is correct, is that evidence of correlation or causation?
- **c.** Design an epidemiological study to test the hypothesis that reduced diversity of the human microbiome is responsible for increasing rates of immune disorders. (Hint: Refer back to Chapter 1 for observational and epidemiological studies.)

Average Number of Microbial Groups in Different Human Populations



Data from Yatsunenko et al. 2012. Human gut microbiome viewed across age and geography. *Nature* 486: 222–228

Mini Case

Apply Your Knowledge

21. Your friend's mother has been hospitalized and has developed a *Clostridium difficile* (*C. diff*) infection of the colon. This is a common hospital-associated infection, in which the use of antibiotics to treat another infection reduces the normal microbiome of the colon, allowing *C. diff* to establish itself. Her doctor is recommending a fecal microbial transplant (FMT), in which feces from a healthy donor are transferred into the intestinal tract of a person with *C. diff* (either by enema or by a tube extending from the nose to the small intestine). Your friend and her mother find this idea to be disgusting, particularly when they realize that the donor fecal material is not sterilized before the transplant.

Data from a clinical trial comparing FMT to standard antibiotic therapy with vancomycin are shown in the two bar charts.

a. What do you observe about the microbial diversity in patients versus donors?

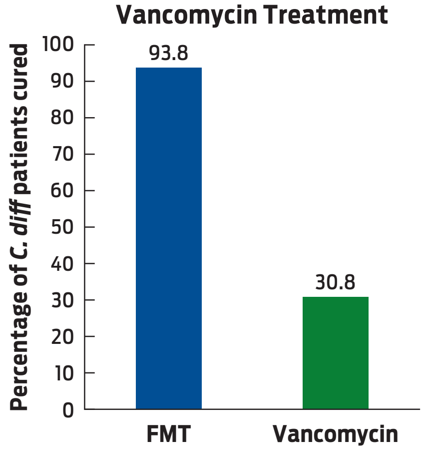
b. What do you observe about the microbial diversity in patients before and after treatment?

c. What can you infer about how the FMT works?

d. Based on your answer to part c, why can't the donor fecal material be sterilized?

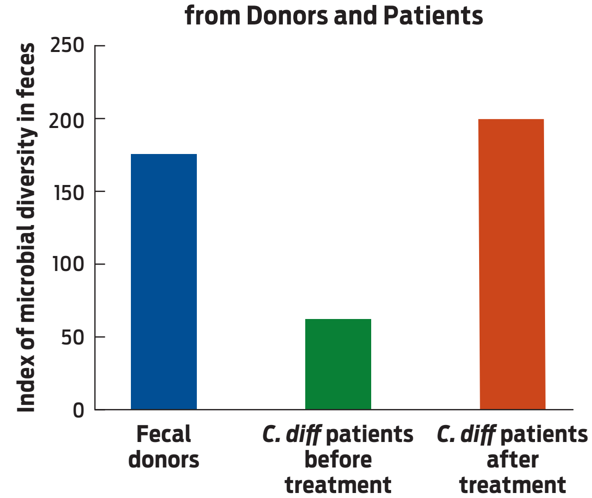
e. Based on the data presented, what treatment do you encourage your friend's mother to choose? Explain your answer.

Cure Rates for FMT and Vancomycin Treatment



Data from van Nood et al. 2013. Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*. *NEJM* 368(5):407–415.

Microbial Diversity in Fecal Samples from Donors and Patients



Data from van Nood et al. 2013. Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*. *NEJM* 368(5):407–415

Bring It Home

Apply Your Knowledge

22. Your friend is thinking of starting an extreme, animal-based keto diet—a diet emphasizing meat, eggs, and cheese. Your friend has heard that keto diets can result in rapid weight loss and loves meat and cheese. You have heard that diet can alter the gut microbiome. You do a quick search and identify a study in which participants ate an animal- or plant-based diet, then the diversity of the gut microbiome was analyzed (both in terms of numbers of different groups and the specific microbes present). People following the animal-based diet experienced a large change in their gut microbiome, including a large increase in the number (and proportion) of bile-resistant bacteria. In mice, these bile-resistant bacteria can cause inflammatory bowel disease. Explain to your friend why this diet may have some side effects (beyond nutritional concerns and weight loss).

Source: L. A. David et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559–563. doi:10.1038/nature12820